Invitrogen
life technologies

LIBRARY EFFICIENCY® DH5α™ Competent Cells

Cat. No. 18263-012

Size: 1 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:
LIBRARY EFFICIENCY DH5α Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells are suitable for cloning experiments using limiting amounts of DNA. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the ß-galactosidase gene from pUC or similar vectors and, therefore, can be used to produce blue/white screening of colonies on bacterial plates containing X-gal or Bluo-gal. DH5α is capable of being transformed efficiently with large plasmids, and can also serve as a host for the M13mp cloning vectors if a lawn of DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101 or JM107 is provided to allow plaque formation.

Component	Part No.	Amount per Vial
DH5α™ Competent Cells	98263	200 µl
pUC19 DNA (0.01 µg/ml)	95340	100 µl

LIBRARY EFFICIENCY DH5α Competent Cells yield > 1 x 10^8 transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of control pUC19 (25 ng) generate > 1 x 10^5 ampicillin-resistant colonies in a 100-µl reaction.

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Transformation Procedure:

A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. To obtain maximum efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on wet ice. Place required number of 17 x 100 mm polypropylene tubes (Falcon® 2059; see Note 1) on wet ice.
2. Gently mix cells, then aliquot 100 µl competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency, add 5 µl (50 pg) control DNA to one tube containing 100 µl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. For DNA from ligation reactions, dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1 µl of the dilution to the cells (1-10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.
9. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544-034).
10. Shake at 225 rpm (37°C) for 1 hour.

1. Dilute the reaction containing the control plasmid DNA 1:10 with S.O.C. Medium. Spread 100 µl of this dilution on LB or YT plates with 100 µg/ml ampicillin and 50 µg/ml X-gal (Cat. No. 15520-034) or Bluo-gal.
2. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution as described in Step 11.
3. Incubate overnight at 37°C.

Growth of Transformants for Plasmid Preparations:

DH5α Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB(2). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes:

1. Falcon 2059 tubes or other similarly shaped 17 x 100 mm polypropylene tubes are required for optimal transformation efficiency. Microcentrifuge tubes (1.5 ml) can be used but the transformation efficiency will be reduced 3- to 10-fold.
2. LIBRARY EFFICIENCY DH5α Competent Cells are refreezable. Subsequent freeze-thaw cycles will reduce transformation efficiency approximately 2-fold.
3. Media other than S.O.C. Medium can be used but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold.

4. LIBRARY EFFICIENCY DH5α can support the replication of M13mp vectors. However, DH5α is F^- and cannot support plaque formation. Therefore, log-phase DH5α-FT, DH5αF', DH5αF'IQ, JM101 or JM107 cells must be added to the top agar which should contain X-gal (Cat. No. 15520-034) or Bluo-gal, final concentration 50 µg/ml and IPTG (Cat. No. 15529-019), final concentration 1 mM. The competent cells should be added to top agar after lawn cells, IPTG and Bluo-gal or X-gal have been added. Incubation at 37°C for 1 hour is not required after addition of S.O.C. Medium.
5. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 50 pg pUC19 yields 100 colonies when 100 µl of a 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 2 \times 10^8$$

References:

1. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Tartof, K. D. and Hobbs, C. A. (1987) *FOCUS® 9:2*, 12.